tial record allows us to estimate the maximum rate of relaxation which we designate S. Its mean value is 3.4 kg/sec in normal boys and 0.72 kg/sec in the patients. Since S is no doubt influenced by the absolute force developed by the corresponding contraction, we rather consider the relaxation speed/kg as given by the ratio S:P. The mean ratios are 10 in normals and 6.5 in the dystrophics. They become equal at 8.4 for the 200th twitch (Table).

In conclusion the dystrophic disorder produces characteristic changes in the capability for tension development and for staircase potentiation in skeletal muscle. These contractile anomalies appear at a preclinical stage when the electrical responses of the muscle still appear normal[5]. In the present paper we emphasize that the twitch kinetics undergoes rather little changes in human dystrophic muscle. Our study refers to the rested muscle studied in situ, at 37–38 °C, under optimum initial tension and with supramaximal stimulation of the motor nerve. Under these conditions the twitch relaxation time is slightly prolonged (+ 23%) and the relaxation speed/kg is reduced (− 35%). However the 2/sec stimulation eliminates these differences as the relaxation accelerates more in dystrophic muscle than in normal muscle (Table). These findings for human muscle are at variance with those reported for dystrophic mouse muscle in vitro in which relaxation was 3 times slower than in controls[11]. Our results suggest that the $Ca^{++}$ sequestration process governing relaxation[12] in the sarcomeres is not irreversibly damaged by Duchenne muscular dystrophy. The observations on contraction times also suggest that the myosin ATPase has normal activity in the still excitable dystrophic muscle fibres[13].

| | Normal boys (6) | Duchenne dystrophy (15) | *t*-test |
|---|---|---|---|
| $CT_1$ (msec) | 76 ± 4 | 85 ± 11 | $P = 0.06$ |
| $CT_{200}$ | 69 ± 5 | 73 ± 8 | $P = 0.25$ |
| $T^1/_2R_1$ (msec) | 61 ± 7 | 75 ± 9 | $P < 0.01$ |
| $T^1/_2R_{200}$ | 58 ± 11 | 61 ± 8 | $P = 0.5$ |
| $S_1:P_1$ | 10 ± 1.2 | 6.5 ± 1.4 | $P < 0.01$ |
| $S_{200}:P_{200}$ | 8.4 ± 2.3 | 8.4 ± 1.4 | $P = 0.9$ |

*Résumé.* Les propriétés mécaniques du muscle adducteur du pouce atteint de dystrophie musculaire de type Duchenne ont été étudiées in situ chez 15 malades. La force produite par la secousse isométrique et le tétanos isométrique est réduite dans tous les cas. Le temps de contraction de la secousse isométrique et son temps de relaxation sont légèrement supérieurs aux valeurs de contrôle. Cette différence disparait au cours de la stimulation répétée du nerf à 2/sec. Ces observations montrent que l'activité ATPase de la myosine (déterminant la vitesse de contraction) et le processus de séquestration du $Ca^{++}$ myoplasmique dans le réticulum sarcoplasmique (déterminant la vitesse de relaxation) ne sont pas altérés de façon irréversible dans la dystrophie musculaire de Duchenne.

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[12] R. J. Podolsky and L. L. Costantin, Fedn Proc. Fedn Am. Socs exp. Biol. *23*, 933 (1964).

[13] This work was supported by the Muscular Dystrophy Associations of America Inc. and by the Fonds de la Recherche Scientifique Médicale.

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## The Effect of Partial Hepatectomy upon Circadian Distribution of Mitosis in the Cornea of Rats

The stimulation of mitosis which occurs in the cornea of rats following partial hepatectomy has been intensively investigated as to its relation to physiological mechanisms which control cell division[1].

The stimuli are apparently related to adrenal gland function because, while it is absent in adrenalectomized rats, it is restored in dexamethasone treated adrenalectomized rats[2]. Whether this stimulating action results from direct or indirect glucosteroid effect upon the metabolism of corneal epithelial cells is not known at the present time[3].

In previous work, the occurrence of cells in mitosis in the cornea of normal and in partially hepatectomized rats was determined in only 2 time periods of the day i.e. 10.00 and 23.00, and thus afforded a partial view only of the diurnal distribution of mitotic activity[3]. Therefore, additional data were sought in order to obtain: (1) a truly circadian distribution of mitosis: (2) a more complete picture of the first 24 h period post partial hepatectomy, in which, according to previous data, increased mitotic activity was not present and the absence of which was thought to be related to either post surgery fast, stress and/or displacement of the morning mitotic peak to some other time period of the day. Thus the different groups of partially hepatectomized rats were sacrificed at 03.00, 07.00, 10.00, 15.00, 19.00 and 23.00 of the day. The control levels of mitosis for the different periods of the day were obtained in 2 separate experiments carried out with 1 month interval between them. The possibility that significant physiological variations in the levels of mitosis could occur from day to day, in spite of the apparent maintenance of environmental conditions, was thus verified.

Female rats of the Wistar strain, with weight ranging between 120–140 g, were used in all experiments. Partial hepatectomy was performed according to the technique of Higgins and Anderson[4]. The animals were sacrificed

[1] K. E. Pashkis, Cancer Res. *18*, 981 (1958).

[2] S. S. Cardoso, A. L. Ferreira, A. C. M. Camargo and H. Caldo, Proc. Soc. exp. Biol. Med. *124*, 1142 (1967).

[3] S. S. Cardoso and A. L. Ferreira, Proc. Soc. exp. Biol. Med. *125*, 1254 (1967).

[4] G. M. Higgins and R. M. Anderson, Archs Path. *12*, 186 (1931).

by decapitation at the different times of the day indicated in the Table; the eyes were removed and fixed in a mixture containing alcohol, formaline and acetic acid, stained and mounted in glass slides as described in previous work[2]. Mitosis were counted with the use of a microscope with a reticulum (8 × 8 mm) ocular (× 8) and oil immersion lens (× 100). 50 (125 × 125 $\mu$) fields were counted in each cornea. All phases of mitosis were included but an arbitrary elimination was done of those cells in early prophase and later telophase. All animals were received and kept in the department's animal quarters for 1 week prior to their use in the experiments, on a commercial diet ad libitum and a natural regimen of day light and temperature.

In 2 control experiments, groups of 5 rats each were sacrificed at 15.00, 19.00, 23.00, 03.00, 07.00 amd 10.00 (Table). In a third experiment hepatectomy was carried out in a group of 75 normal rats, between 09.00 and 11.00. The sacrifice of each group of 5 rats started at 19.00 of the same day in which hepatectomy was performed; the subsequent groups were sacrificed at 4 h intervals up to 52 h post hepatectomy. The results obtained both with normal and partially hepatectomized rats are presented in the Table.

Circadian distribution of mitosis in the cornea of normal and partially hepatectomized rats

| Time of day | Mitosis/50 high power microscopic fields ± S.E.[a] | | | |
|---|---|---|---|---|
| | Control[b] | Control II | 70% hepatectomized | |
| 19.00 | 16 ± 6.9 | 9.4 ± 5.1 | 47 ± 8.3 | (8 $P < 0.02$)[c] |
| 23.00 | 26 ± 3.4 | 30 ± 2.6 | 104 ± 30 | (12 $P < 0.02$) |
| 03.00 | 101 ± 20 | 114 ± 9.5 | 121 ± 32 | (16 $P > 0.05$) |
| 07.00 | 228 ± 28 | 224 ± 21.8 | 191 ± 46 | (20 $P > 0.05$) |
| 10.00 | 172 ± 6.9 | 176 ± 14 | 148 ± 42 | (24 $P > 0.05$) |
| 15.00 | 108 ± 2 | 125 ± 13.6 | 197 ± 21 | (28 $P < 0.005$) |
| 19.00 | | | 16 ± 4 | (32 $P > 0.05$) |
| 23.00 | | | 148 ± 37 | (36 $P < 0.02$) |
| 03.00 | | | 194 ± 22 | (40 $P < 0.02$) |
| 07.00 | | | 396 ± 87 | (44 $P > 0.05$) |
| 10.00 | | | 246 ± 13 | (48 $P < 0.01$) |
| 15,00 | | | 132 ± 25 | (52 $P > 0.05$) |

Time of day, the hour in which the rats were sacrificed; [a] S.E., standard error of the mean values; [b] two separate control experiments carried out with 1 month interval; [c] hours post partial hepatectomy and $P$ values when compared to normal controls.

The results obtained with the 2 control groups indicate that no significant day to day variations occur in the number of cells in mitosis when comparing control animals sacrificed at comparable time periods of the day.

The results also show that the general distribution of mitosis throughout the day is practically the same in both the normal and in hepatectomized animals, with a period of minimal occurrence of mitosis at 19.00 and a peak at 07.00. While the general circadian distribution of mitosis is practically the same in both groups, statistically significant higher levels of mitosis were found in several of these groups of partially hepatectomized rats, when compared to their normal controls. The $P$ values for the groups sacrificed at the different hours post partial hepatectomy were as follows: 8 h, $P < 0.02$; 12 h, $P < 0.02$; 16 h, $P > 0.05$; 20 h, $P > 0.05$; 24 h, $P > 0.05$; 28 h, $P < 0.005$; 32 h, $P > 0.05$; 36 h, $P < 0.02$; 40 h, $P < 0.02$; 44 h, $P > 0.05$; 48 h, $P < 0.01$ and 52 h, $P > 0.05$, when compared with their respective (hour of the day at which the control animals were sacrificed) normal controls.

In summary, these results indicate that the mechanism(s) which synchronize the occurrence of mitosis in the cornea of normal animals is still active in hepatectomized rats as: (1) the periods of the day in which maximal and minimal numbers of cells in mitosis occur in the corneas of partially hepatectomized rats and in control rats is similar, as also is the overall distribution of mitosis; (2) that increased mitotic activity is found particularly in the second day following partial hepatectomy[5].

*Zusammenfassung.* Die Resultate zeigen, dass der oder die Mechanismen, welche die Mitosis in der Cornea von normalen Tieren synchronisieren, nach partieller Hepatektomie immer noch aktiv sind.

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[5] This work was supported in part by a Grant (CY-6473) from the Natl. Inst. Health, USPHS, and from Fundacao de Amparo à Pesquisa do Estado de Sao Paulo, Brazil.

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## Pancreozymin/Cholecystokinin a Physiological Mediator of Gastric Secretory Inhibition of Duodenal Origin

The most highly purified form of pancreozymin/cholecystokinin (PZ/CCK)[1] will stimulate gastric acid secretion and potentiate the stimulatory action of methacholine on gastric acid. On the other hand PZ/CCK antagonizes gastric acid secretion which has been stimulated by feeding or by i.v. gastrin pentapeptide[2,3].

If this is a physiological property of PZ/CCK then endogenously released hormone should manifest the same actions. We have demonstrated that this is the case in conscious dogs with Heidenhain gastric pouches. Four of these had duodenal fistulae in addition and 3 had Thiry Vella loops of duodenum. Gastric secretion was stimulated with a continuous i.v. infusion of either methacholine or gastrin pentapeptide (Ayerst Laboratories). In every case when methacholine was the gastric acid stimulant, the

[1] E. JORPES and V. MUTT, Acta physiol. scand. *66*, 196 (1966).
[2] D. F. MAGEE and M. NAKAMURA, Nature *212*, 1478 (1966).
[3] J. C. BROWN, L. P. JOHNSON and D. F. MAGEE, Gut *8*, 29 (1967).